GREAT SHIPS INITIATIVE (GSI) STANDARD OPERATING PROCEDURE (SOP) DEVIATION FORM

DATE/TIME: 9/4/2009 to 9/9/2009 (Form Completed 10/26/2009)
TEST ID NUMBER: 09-SI-3
RDTE FACILITY OR BENCH-SCALE TESTING? Research, Testing, and Evaluation Facility Test
GSI RESEARCH TEAM MEMBER NAME/TITLE: Kelsey R. Prihoda, GSI Assistant QA/QC Officer

Deviation Number	Description of Deviation (Include SOP Number and Title)	Detailed Description of Impact on Study (If Any)	Description of Corrective Actions Taken (If Needed)
1	SOP No: GSI/SOP/BS/RA/MA/3 – Procedure for the Detection and Enumeration of *Enterococcus* using Enterolert. Section Quanti-Tray Enumeration Procedure, ¶12 Quanti-Tray results for treatment samples were read at 22.75 hours (fill samples) and 22.5 hours (discharge samples) after the Quanti-Trays were placed in the incubator. Enterolert results are definitive at 24-28 hours; however, all positives read prior to 24 hours are valid.	The impact of this deviation on Trial 3 cannot be determined, as a second count was not done 24-28 hours after incubation (when results are definitive). It is unlikely that the *Enterococcus spp.* densities would have increased with the additional 1.25 or 1.5 hours of incubation time, as sample media was active for more than 24 hours.	No corrective action taken at the time of the deviation. Future analyses should be conducted according to SOPs.
2	SOP No: GSI/SOP/BS/RA/MA/4 – Procedure for the Detection and Enumeration of Total Coliforms and *E. coli* using IDEXX's Colilert. Section Quanti-Tray Enumeration Procedure, ¶11. Quanti-Tray results for treatment samples were read at 22.75 hours (fill samples) and 22.5 hours (discharge samples) after the Quanti-Trays were placed in the incubator. Colilert results are definitive at 24-28 hours; however, all positives read prior to 24 hours are valid.	The impact of this deviation on Trial 3 cannot be determined, as a second count was not done 24-28 hours after incubation (when results are definitive). It is unlikely that the the *E. coli*/Total Coliform densities would have increased with the additional 1.25 or 1.5 hours of incubation time, as the sample media was active for more than 24 hours.	No corrective action taken at the time of the deviation. Future analyses should be conducted according to SOPs.

3	SOP No.: GSI/SOP/RO-E/SA/M/3 – Procedure for the Colony Blot Preparation for the Enumeration of Cultured Vibrio cholerae (DRAFT). Section RNA Colony Blot Preparation ¶19-21. RNA membranes were not exposed to UV light until the end of the blot procedure. Prior to storage, membranes are dried for 10 min. at 80°C, exposed to UV light for 1 min., and baked for 15 min. at 70°C.	The impact of this deviation on Trial 3 is not known.	No corrective action taken at the time of the deviation. Future analyses should be conducted according to SOPs.

GSI Research Team Member Comments: No further comments regarding this deviation.

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GREAT SHIPS INITIATIVE (GSI) STANDARD OPERATING PROCEDURE (SOP) DEVIATION FORM

DATE/TIME: 9/9/2009 (Form Completed 10/26/2009)
TEST ID NUMBER: 09-SI-3
RDTE FACILITY OR BENCH-SCALE TESTING? Research, Testing, and Evaluation Facility Test
GSI RESEARCH TEAM MEMBER NAME/TITLE: Kelsey R. Prihoda, GSI Assistant QA/QC Officer

Deviation Number	Description of Deviation (Include SOP Number and Title)	Detailed Description of Impact on Study (If Any)	Description of Corrective Actions Taken (If Needed)
1	SOP No: GSI/SOP/BS/RA/RT/6 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*. Section "Test Procedure", ¶11. *C. dubia* were fed half the required volume (15 mL not 30 mL) of Yeast-Cereal Leaves-Trout Chow suspension (YCT) and *Selenastrum capricornutum*. Each feeding must consist of 0.2 mL Yeast-Cereal Leaves-Trout Chow suspension (YCT) and 0.2 mL *Selenastrum capricornutum* concentrate/30 mL exposure solution (to provide 2-2.3 x 105 cells/mL).	There is not an impact on Trial 3 *C. dubia* WET Test as a result of this deviation. This test met the test acceptability criteria for *C. dubia* as set by the US EPA.	No corrective action was taken at the time of the deviation. The correct volume of YCT and *S. capricornutum* were fed to *C. dubia* during Trials 4-7.
2	SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT). Section "Test Procedure", ¶5. Average initial *S. capricornutum* density was 255,889 cells/mL. Each milliliter of inoculum must contain enough cells to provide an initial cell density of approximately 10,000 cells/mL (± 10%) in the test flasks.	At this time it is not clear what the impact on the Selenastrum WET will be. Corrective action will be taken for future trials.	No corrective action was taken at the time of the deviation.

3	SOP No. GSI/SOP/BS/RA/R_v8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga *Scenedesmus capricornutum* (DRAFT), Section "QA/QC" ¶4. There was no QA count conducted during the *S. capricornutum* WET Test. A QA count of the algae cell concentration in at least 10 % of the test chambers must be performed during every trial.	The impact on Trial 3 WET Testing as a result of this deviation is that there is no measurement of operator/counting bias for the *S. capricornutum* WET Test.	No corrective action was taken at the time of deviation. It will be important to conduct QA counts on *S. capricornutum* WET Tests in the future in order to determine an acceptable level of bias.

GSI Research Team Member Comments: No additional comments regarding WET Testing SOP Deviations.

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